ABSTRACT

Our objective was to determine the impact of simultaneous removal of lactose plus low molecular weight solutes and milk serum proteins from skim milk by microfiltration (MF) on the chemical, physical and sensory properties of 3.4, 7.5, and 10.5% milk protein-based beverages before and after a direct steam injection thermal process. Skim milk was microfiltered at 50°C using 0.1 micron ceramic membranes with a diafiltration ratio of water to milk of about 2.5. Milk lactose, serum proteins, and soluble minerals were removed simultaneously to produce protein beverages containing from 3.4 to 10.5% true protein from skim milk and this process was replicated twice with different skim milks. The soluble mineral plus lactose content was very low and the aqueous phase of the beverages had a freezing point very close to water (i.e., −0.02°C). Beverage pH ranged from 7.19 to 7.41, with pH decreasing with increasing protein concentration. Overall, the beverages were whiter and blander than skim milk. When UHT processed with direct steam injection at a holding temp of 140°C for 2 to 3 s, there was some protein aggregation detected by particle size analysis (volume mean diameter of protein particles was 0.16 micron before and 22 microns after UHT). No sulfur/eggy flavor was detected and no browning was observed due to the UHT thermal treatment. Both apparent viscosity and sensory viscosity increased with increasing protein concentration and heat treatment.

Key Words: microfiltration, low lactose and serum protein, milk protein beverage

INTRODUCTION

Consumer demand for protein continues to rise, with the global protein market set to grow to over 114 billion US dollars by 2030 (Shahbandeh, 2023). While dairy protein-based products have seen recent competition from plant-based protein products, they still experience higher popularity due to their nutritional value, good taste, and status as a complete protein (McCarthy et al., 2017). Currently, more consumers are paying attention to declared ingredients and are avoiding foods and beverages with chemical sounding ingredients, creating a purchasing phenomenon known as the clean label trend (IFIC, 2021). Lactose free dairy products are another growing market (Allied Market Research, 2022) with opportunities for beverage innovation to meet this consumer demand (Hernandez et al., 2023).

Two clean label forms of dairy protein isolation, separation and concentration are ultrafiltration (UF) and microfiltration (MF). These technologies use filtration membranes of varying pore size to fractionate milk constituents into products without the need for added ingredients. UF membranes are primarily used in the dairy industry to concentrate proteins to produce whey protein concentrate (WPC) and milk protein concentrate (MPC) while MF membranes are primarily used to separate casein and milk derived whey proteins to produce micellar casein concentrate (MCC) and milk derived whey proteins (MDWP) (Bylund, 2003; ADPI, 2023) or to remove bacteria and spores from milk, whey or combined dairy and juice feed materials (Elwell and Barbano, 2006; Caplan and Barbano, 2013; Vieira et al., 2020). UF does not alter the 80:20 ratio of casein to whey protein within skim milk and thus retains the same casein as a percent of true protein (CN%TP) (80 to 82%). MCC does not retain whey and can contain up to 95% CN%TP (ADPI, 2023; Carter et al., 2021).

Other milk solids constituents removed in the permeate by UF and MF processes include lactose and soluble minerals (calcium, potassium and phosphorous) and nonprotein nitrogen compounds. Both UF and MF membranes can be used to manufacture lactose-reduced or lactose-free beverages because neither process retains lactose (GEA, 2020). Lactose intolerance affects approximately 68% of the world (NIH, 2018), and the lactose-free dairy product market is projected...
to rise from $10.6 billion in 2017 to $17.8 billion by 2027, demonstrating the use for these technologies in this growing market (Shahbandeh, 2018). Recent research has achieved 96% lactose removal from milk using UF with a diafiltration ratio of water to starting feed of approximately 3:1 (Hernandez et al., 2023). They reported that pH and L-value (whiteness) of milk increased with increasing lactose and soluble mineral removal, while titratable acidity and apparent viscosity decreased. Sensory viscosity and yellowness decreased while sensory whiteness and astringency increased with lactose and soluble mineral removal (Hernandez et al., 2023).

MCC and MPC are used as protein ingredients in thermally processed ultra-pasteurized and shelf stable protein beverages. Ultra-high temperature (UHT) pasteurization (both indirect heating and direct steam injection) is used to produce shelf-stable high protein beverages. Previous research has shown that UHT processed dairy products have cooked, sulfur and eggy off flavors (Mehta, 1980; Calvo and de la Hoz, 1992) due to the high heat exposure. More recent studies have reported that serum proteins (SP) are the milk proteins responsible for these off-flavors (Lee et al., 2017; Jo et al., 2018). Sulfur containing amino acids such as cysteine and methionine are the primary origin of volatile sulfur compounds such as hydrogen sulfide, carbon disulfide, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide that contribute to these off flavors (Jo et al., 2018). Jo et al. (2018) also determined that hydrogen sulfide (sulfur/eggy aroma) and carbon disulfide (cooked aroma) were the key sources of sulfur and cooked aromas and flavors in UHT processed milks. Other research has explored the limit of SP removal at which off flavors in UHT processed milks are no longer detectable. Vogel et al. (2019) found no detectable sulfur/eggy off flavor in 95% SP removed UHT MCC beverages. Furthermore, Whitt et al. (2022) reported no detectable sulfur/eggy flavors in UHT MCC beverages with added fat at 91% SP removal. Hurt et al. (2010) estimated that it should be possible to achieve up to 97% SP removal using a 3x, 3-stage ceramic MF membrane process with uniform transmembrane pressure (UTP). The objective of our research was to determine the impact of simultaneous removal of lactose plus low molecular weight solutes and milk serum proteins on the chemical, physical and sensory properties of a 3.4, 7.5, and 10.5% milk protein-based beverages before and after a direct steam injection UHT thermal process.

### Experimental Design

MF at 50°C was used to process pasteurized skim milk (ca. about 3.4% true protein) and simultaneously remove lactose and milk SP. This was done as a batch continuous diafiltration process where as MF permeate was removed, an equal volume of 50°C deionized diafiltration (DF) water was added while the retentate was recirculated directly back to the MF feed tank. This produced a low lactose (ca. 0.2%) and low milk serum protein (ca. 95% removed) MF retentate with the goal in this part of the process to produce an MF retentate at a protein content lower than the original skim resulting due to the retention of casein but removal of the original serum proteins. When this was achieved, the ratio of DF water used to the original amount of skim milk used was about 2.6. Without stopping the MF process, filtration and removal of MF permeate was continued without DF to concentrate the protein to 10.5% (Figure 1). At that point the MF process was stopped and the 10.5% protein MF beverage was collected. Next, a portion of the 10.5% MF beverage was diluted with DI water to 7.5% and another portion to 3.4% true protein concentration. Each of these 3 MF beverages were split into 2 batches: one batch received no further heat processing and the other batch was processed with a UHT direct steam injection (DSI) system with holding temperature and time of 140°C and 2 to 3 s, respectively. A mass-balance for this process and formulation of beverages is presented in Figure 1. Viscosity, color, particle size, calcium, phosphorous, potassium, proximate composition, and sensory properties were analyzed on all batches. The experiment was replicated 2 times starting with 2 different batches of pasteurized skim milk on different days.

### Skim Milk Processing Before Filtration

Raw bovine skim milk was obtained from the North Carolina State University Dairy Enterprise System (Raleigh, NC) the day before each processing run. The raw skim milk was kept and held overnight at 4°C in a stainless-steel glycol chilled jacketed storage tank. On the day of membrane filtration, raw skim milk (approximately 400 kg) was prefiltered at 4°C before pasteurization using a Nexis T-filter (NXT 10–30U-M7S, Pall Corp., Port Washington, NY) to remove foreign material and then pasteurized (870 kg/h) with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC) at 72°C for 15s.
Microfiltration

Pasteurized skim milk (approximately 400 kg) was warmed to 50°C using a plate heat exchanger and microfiltered with a GP MF system (Tetra Alcross MFS-7, TetraPak Filtration Systems) equipped with 0.1-µm nominal pore diameter ceramic Membrolax (model EP1940GL0.1µ.AGP1020, alumina, Pall Corp.) membranes (surface area of 1.7 m², membrane length of 1.02 m). The MF modules had a tubular configuration with 7 ceramic membrane sticks, 19 round channels per stick with a 4-mm channel diameter, in one stainless steel module that was mounted vertically in the system as described by Zulewska et al. (2009). Cleaning was as described by Zulewska et al. (2009).

Skim Milk Microfiltration and Diafiltration

Pasteurized skim milk (ca. 400 kg) was microfiltered and diafiltered at 50°C. At the start of the run, approximately 70 kg of permeate was collected and weighed without diafiltration to increase the starting protein concentration. About 45 kg of permeate was collected weighed and an equal weight of 50°C deionized water was added to the feed tank and mixed. This process was repeated until the total amount of DF water added was about 2.6 times the starting weight of milk. The MF process was run at a 2X concentration factor (weight ratio of permeate to retentate) and flux of about 60 kg/m²/h. Permeate and retentate were collected every 15 min for analysis with an infrared milk analyzer (Lactoscope FTIR model FTA, Delta Instruments, Drachten, the Netherlands) to monitor their composition. For the final concentration the MF retentate was concentrated.
by permeate removal with no DF to achieve a protein content of about 10.5%.

Milk and beverage freezing points were measured using an Advanced Instruments milk cryoscope (Model 4250, Norwood, MA, USA). The pH of each formulation was determined at 20°C using a pH meter (Fisher Scientific, Accumet, Model 915) and gel filled electrode (Mettler-Toledo HA-405 DXK-S8/120, Columbus, OH). The pH meter was calibrated using a pH 7 and 4 buffers at 20°C (Fisher Scientific).

**Beverage Formulation for Thermal Processing**

The MF retentate containing 10.5% true protein was diluted with DI water by weight to make 2 additional batches of beverage, one at 7.5% and one at 3.4% protein. Each of the 3 batches of lactose and milk serum protein reduced beverages were split in half and one portion was not heat processed and the other was heat processed. There were no flavor or emulsifying salts added to the beverages.

**Thermal Processing: Direct Steam Injection**

The beverages were thermally processed in a manner similar to Lee et. al. (2017), using a Microthermics EHVH pasteurization unit (Microthermics Inc., Raleigh, NC) running with T12B software (10.11.12.90, v6.0, build 104) with a 2-stage homogenizer (model NS2006H, GEA Niro Soavi, Parma, Italy). The beverages were continuously fed to a Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) at a flow rate of 1.4 L/min, and then UHT processed by direct steam injection (DSI): preheated to 90°C, pasteurized at 140°C for 2 to 3 s under 330-kPa pressure with culinary DSI (model LG-30, Electro-Steam Generator Corp., Alexandria, VA), cooled via vacuum chamber at –432 mmHg (–57.6 kPa) of vacuum, and homogenized using a 2-stage in-line homogenizer (GEA Niro Soavi, Parma, Italy). The vacuum applied to the milk + steam mixture reduced the temperature of the milk at the outlet of the vacuum chamber to a temperature of about 87°C, which was about 3°C lower than the preheat temperature. The beverages were then homogenized (second stage 3450 kPa, total 17,240 kPa) using a 2-stage in-line homogenizer (GEA Niro Soavi, Parma, Italy) post DSI treatment.

**Chemical Analyses**

Beverages were analyzed in duplicate for TS, total N (TN), noncasein nitrogen (NCN), and nonprotein nitrogen (NPN) content using forced-air oven drying (AOAC, 2019; method 991.20), Kjeldahl TN (AOAC, 2019; method 991.20), Kjeldahl NCN (AOAC, 2019; method 990.05) and Kjeldahl NPN (AOAC, 2019; method 991.21), respectively. The TP was calculated by subtracting NPN from TN and multiplying by 6.38; CN (casein) was calculated by subtracting the NCN from TN and multiplying by 6.38; and whey protein content was calculated by subtracting NPN from NCN and multiplying by 6.38.

**Microbiological Analyses**

Beverages were tested in duplicate after processing for aerobic plate count (Laird et al., 2004; 6.040) and coliform count (Davidson et al., 2004; 7.071) using Petrifilm Aerobic Count Plate, 3M ID 7100039310 and Petrifilm Coliform Count Plate, 3M ID 7100039392 (3M Food Safety, Maplewood, Minnesota) to determine microbial quality.

**Viscosity and Color Analysis**

Apparent viscosity (AV) was measured (Adams and Barbano, 2015) using a rotational viscometer (LV-DV2T, Brookfield Engineering Laboratories Inc., Middleboro, Massachusetts) equipped with a jacketed cup-and-bob fixture (Enhanced UL Adapter, Brookfield Engineering Laboratories Inc.). The calibration of the viscometer (LV-DV2T, Brookfield Engineering Laboratories Inc., Middleboro, Massachusetts) was checked at 25°C over a range of torques (about 10, 30, and 70%) before use using a Microsoft Excel calibration template (Brookfield Engineering Laboratories Inc., 2021), a pair of general purpose silicone standards (cP [5 mPa·s] and 100 cP [100 mPa·s], Brookfield Engineering Laboratories Inc., Middleboro, Massachusetts). Measurements for each of the 3 different protein beverages were conducted at 4°C on the beverages (3 no heat treatment and 3 DSI) as described below. The beverages were tempered at 4°C in a circulating refrigerated water bath (PolyScience, SD7LR-20, Warrington, PA) for 5 min to allow the samples to equilibrate.

Color of milk beverages was determined in duplicate using an Ultra Scan Pro Spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA) at 4°C. A water bath (PolyScience, SD7LR-20, Warrington, PA) was used to maintain sample temperature at 4°C. Beverages were measured in reflectance mode, wavelength from 360 to 750 nm with a 5 nm resolution, using Illuminant A at 10 degree viewer angle and reflectance curves were recorded, as described by Cheng et al. (2018). The cuvette was glass with a 20 mm path length. Color data were collected and Hunter L, a, and CIE b*-values were reported as recommended for dairy products (Cheng et al., 2018).
Particle Size Analysis

Beverages were analyzed using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Enigma Business Park, Malvern, Worcestershire, UK, software version 5.4) as described by Di Marzo et al. (2016). Refractive indices of 1.57 for the protein particles and 1.33 for the water (42°C) suspending liquid were used for a range of particle size from 0.02 to 2000 µm. The Malvern multiple narrow mode model for spherical particles was used. The measure time for sample and background was set at 5 s with 5,000 snaps. A light obscuration range limit was set to fall with a range of 7 to 9%, with 3 measurement cycles per sample with a zero-time delay between measurements.

Mineral Analysis

Calcium, potassium, and phosphorus were measured in duplicate by DairyOne Laboratory (Ithaca, NY) on beverage samples at the beginning of storage. Samples were held at −80°C for storage then moved to a −20°C freezer the day before analysis. Samples were removed from the −20°C freezer and placed for 30 min into a 40°C water bath, then mixed gently by inversion for homogeneity before weighing for analysis. Samples were digested using the CEM Microwave Accelerated Reaction System (MARS6) with MarsXpress Temperature Control using 50 mL calibrated Xpress Teflon PFA vessels with Kevlar/fiberglass insulating sleeves then analyzed by Inductively Coupled Plasma (ICP) using a Thermo iCAP 6300 or iCAP Pro XP Inductively Coupled Plasma Radial Spectrometer. Samples (about 5 g) were first pre-digested at ambient temperature for 10 min with 8 mL nitric acid (HNO₃) and 2 mL hydrochloric acid (HCl) and then an additional 10 min with 1 mL 30% hydrogen peroxide (H₂O₂). After pre-digestion was completed, samples were digested in 2 stages: Stage one had a 10 min ramp to 135°C and held for 3 min at 1500W. Stage 2 had a 12 min ramp to 200°C and held for 15 min at 1600W. Vessels were brought to 50 mL volume, with an aliquot used for ICP analysis. The following calibration reference standards (Assurance Spex Certiprep Stock Standards 203 Norcross Avenue Metuchen, NJ 08840) were used: Calcium 10,000 µg/mL in 5% HNO₃ – Catalog# PLCA2–3X; Phosphorous 10,000 µg/mL in H₂O – Catalog# PLP9–3X; Potassium 10,000 µg/mL in 5% HNO₃ – Catalog# PLK2–3X.

Sensory Analysis

Descriptive analysis was conducted in accordance with the North Carolina State University Institutional Review Board for the Protection of Human Subjects in Research regulations. The protein beverages were evaluated for opacity, whiteness, overall aroma, sweet aromatic, cooked/milky, sulfur/eggy, sweet taste, astrigent mouthfeel, viscosity and chalkiness (Whitt et al., 2022). Seven panelists (4 males, 3 females, ages 21 to 50 y) evaluated each beverage in duplicate. Each panelist had more than 50 h of descriptive analysis experience with milk, milk products, and dairy protein beverages using the Spectrum™ method with a 0 to 15 point intensity scale (Meilgaard et al., 2007). Samples were prepared with overhead lights off to prevent light oxidation. Thirty mL of each beverage were poured into 59-mL souffle cups (Dart Container Corp., Mason, MI) with randomized 3-digit blinding codes and lidded. Beverages were evaluated at 4°C. Data was collected electronically on the NCSU secure network.

Statistical Analysis

Data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Proc GLM of SAS was used to determine the statistical differences (P < 0.05) among means of the 6 treatments (3 protein concentrations each with and without heat treatment). Proc GLM of SAS was used to determine the effects of independent category variables heat treatment (no heat, DSI), protein concentration (3.4, 7.5, and 10.5%) and replicate (n = 2) on dependent variables apparent viscosity, color (L, a, and b² values), and sensory characteristics and their interactions were included in the SAS GLM model. If the F-value for the full model was significant (P < 0.05), then significance (P < 0.05) of each independent variable and its interactions were determined. Principal component analysis (PCA) was conducted on trained panel means using the correlation matrix with XLSTAT 2021 (Addinsoft). All analyses were performed at 95% confidence (P < 0.05).

RESULTS AND DISCUSSION

MF Processing

The average flux during MF at 50°C was 58.63 ± 1.38 Kg/m²/h. Diafiltration with 50°C DI water was used to remove most of the lactose and serum protein from the skim milk with the mean percent removal with amount of DF water shown in Figure 2. It took a DI water volume of about 2.6 times the starting milk volume to achieve 97 to 98.5% lactose removal and a 93% serum protein removal at 50°C with 0.1 micron ceramic MF membranes.
Retentate freezing point, pH, and Titratable acidity during MF processing

The change in freezing point and pH of the MF retentate are shown in Figure 3. As lactose and soluble minerals were removed from skim milk by MF with diafiltration, the freezing point of the MF retentate increased and so did the pH. The final freezing point of the MF retentate was close to that of water (i.e., −0.02°C) and the pH was about 7.4 at 20°C instead of the typical freezing point and pH of milk (i.e., 6.6), even though the retentate contained 3.4 protein. This trend is consistent with previous literature that found lactose and soluble mineral removal increased pH from 6.6 to 7.4, changed the freezing point to be closer to that of pure water, and decreased titratable acidity (TA) over the course of processing lactose free UF retentate (Hernandez et al., 2023). Both the freezing point and pH of the MF retentate increased with diafiltration during MF. Freezing point is a rapid testing method using a milk cryoscope that is commonly found in dairy plant quality control laboratories to test farm milk for adulteration with water, however the same instrument could be used to monitor the removal of lactose and low molecular weight solutes during filtration.

Beverage Composition

The actual protein and total solids concentration achieved for the targeted 3.4, 7.5, 10.5% protein beverages are shown in Table 1. The casein purity (i.e., casein as a percent of true protein) of the beverages averaged about 91 to 93.3% (Table 1), which reflects a high percentage of serum protein removal. Our findings are consistent with previous research (Whitt et al., 2022) that achieved a casein purity of 93.83%. The final pH of the milk protein beverages (Table 2) was much higher than the pH of starting skim milk (i.e., 6.65 at 20°C), which agrees with Hernandez et al. (2023) that reported a final pH of lactose free UF retentate to be 7.4 after processing. For the final beverages, the pH decreased ($P < 0.05$) with increasing protein concentration (7.41, 7.26, 7.19) for 3.4, 7.5, and 10.5% protein beverages, respectively. The original calcium concentration and ratio of Ca/P in the starting skim milk were 1373 mg/1000g of milk and 1.31, respectively. The concentration of calcium, phosphorous, and potassium all increased as protein concentration increased ($P < 0.05$) and the ratio of Ca/P also increased ($P < 0.05$) (Table 2). The 7.5% and 10.5% protein beverages contained 1.94 and 2.65 times more calcium per serving (240 g) than a serving skim milk and this was similar to the report of Hoyt et al. (2023) where they found 7.5% MCC to contain 1.87 times more calcium per serving (240g) than a serving a skim milk. The calcium concentration was highly correlated to increasing protein concentration due to protein bound calcium. The original pH of the skim milk was 6.65 at 20°C and removal of lactose and low molecular weight milk components by filtration caused milk pH to increase and milk TA to decrease (Table 2). The TA of the MF retentate was continuously decreasing during MF/DF (Figure 4). In the final beverages with lactose and low molecular weight soluble components removed, as milk protein content decreased, the pH increased to as high as 7.41 at 20°C ($P < 0.05$) and TA decreased to as low as 0.04% ($P < 0.05$). The amount of decrease in TA was large (from 0.17 to 0.04) and reflected the influence of the removal of milk soluble minerals on...
TA, while the protein bound minerals increased with increasing protein concentration (Table 2). A similar decrease in TA and increase of pH of UF retentate was also reported by Hernandez et al. (2023) for lactose and soluble mineral removal.

**Beverage Particle Size**

The beverages were produced from skim milk with a fat of <0.1%, therefore the concentration of fat droplets was low and the concentration of casein micelles was high in the beverages. The laser light scattering particle size distribution for the 3.4, 7.5, and 10.5% protein beverages without and with UHT-DSI thermal treatment are shown in Figure 5. The particle size distribution of all the unheated 3.4, 7.5, and 10.5% beverages (Figures 5A, B and C) were about the same and had a large peak of particles with a volume mean diameter at about 0.15 microns (casein micelles) and a small peak for fat globules at about 0.8 microns. The volume mean diameter D (4,3) was about 0.16 microns and 22 microns after UHT for all protein concentrations. There was a very strong impact of heat treatment \((P < 0.05)\) on particle size with 98.5% of the variation in D (4,3) being explained by heat treatment and <1% of variation \((P < 0.05)\) was explained by variation in protein concentration (Table 3). The particle size distribution changed as a result of the UHT-DSI heat treatment.
(Figures 5 D, E, and F) which indicated that there was heat induced aggregation of casein micelles, with a range of particles from 1 to about 100 microns in the UHT processed beverages. Hoyt et al. (2023) reported protein aggregates with particle size distribution modes at 0.75 and 10 microns in UHT-DSI 7.5% protein MCC beverages in comparison to HTST processed beverages. Dalgleish (1990) reported that heat during thermal processing causes aggregation of milk protein due to interaction of κ-casein with whey proteins, particularly β-lactoglobulin, to form protein aggregates.

During the pilot scale UHT-DSI thermal process, visible flecks of protein aggregates were observed in the vacuum chamber that was consistent with the aggregation measured in the particle size analysis. Dring the thermal process of 140°C for 2 to 3 s, the proteins in solution are exposed to temperatures in the range of 155 to 160°C at the steam injector tip, so it is not surprising that the high temperature caused protein aggregation. Pranata et al. (2023) concluded that UF and MF beverages at 7.5% protein containing a residual lactose concentration of 0.6 to 0.7% and the proportional amount of soluble milk minerals were heat stable in UHT-DSI processing without addition of dipotassium phosphate. Therefore, when using UF or MF to make lactose free beverages, the total removal of soluble mineral is likely to negatively affect heat stability during UHT processing. Truong et al. (2024) used UF to achieve removal of lactose and soluble minerals from skim milk at 3 different protein beverage concentrations (3.4, 7.5, and 10.5%) and these beverages were thermally processed. Truong et al. (2024) reported that beverages were stable by HTST (75°C for 15s) and in an autoclave at 116°C for 6 min, but not (product coagulated) in UHT-DSI at 142°C for 2 to 3 s, while similar MF beverages produced with very low lactose and soluble mineral in the current study did not coagulate in the UHT-DSI, probably because the MF beverages contained very little heat labile milk serum protein.

### Beverage Apparent Viscosity

The AV of the beverages increased \((P < 0.05)\) with protein concentration and there was a protein by thermal process interaction \((P < 0.05)\), with apparent viscosity increasing with increasing protein concentration and with thermal treatment, particularly at high protein concentration (Figure 6). Differences in AV in our study were primarily driven by protein concentration, which explained about 92% of the variation in AV (Table 3) with protein by heat treatment interaction being apparent for the comparison of the AV of the NH 10.5% protein with the AV for the UHT-DSI treatment (Figure 6). Pranata et al. (2023) reported that amount of migration of casein and bound mineral out of casein micelles in milk protein will impact viscosity and that the use of added salts (e.g., dipotassium phosphate) impacted the movement of casein and calcium out of the casein micelles. Dunn et al. (2021) reported a strong protein x thermal process interaction \((P < 0.05)\) in MCC containing 6.5 to 13.2% protein. However, our viscosity results for 10.5% protein MCC at a temperature of 4°C were almost double (46 mPas vs 25 mPas) the values reported by Dunn et al. (2021). The MF process in Dunn et al. (2021) was a 3-stage 3 X process (i.e., first stage 3X MF with no water added, second and third stage 3X DF) with a total DF ratio of DF water to milk of 1.32, which is lower than the DF ratio in the current study (i.e., about 2.5). The 3X 3 stage process used by Dunn et al. (2021) would produce an MCC retentate with higher lactose and soluble mineral content in the final MF retentate than the continuous diafiltration used in the current study. The difference in soluble mineral content would be expected to produce a
difference in AV between the 2 studies. Similarly, Hoyt et al. (2023) used a 3X, 3 stage process that would produce a final retentate with higher lactose and soluble mineral content and an AV of 21 mPa·s in 7.5% protein MCC at 4°C, which was also lower than the AV observed in the current study. Also consistent with our findings, Ding et al. (2023) documented UHT-DSI processed milk (containing both casein and whey proteins) to have significantly \( P < 0.05 \) higher viscosity than raw, HTST, and UHT-IND processed milks.

**Beverage Color**

The mean \( L, a, \) and \( b^* \) values for the skim milk before MF were 77.98, −4.09, and 2.39, respectively. The \( L, a, \) and \( b^* \)-values for the thermally processed

**Figure 5.** Laser light scattering particle size distribution for low lactose, low serum protein beverages at 3.4, 7.5, and 10.5% protein with no heat treatment and after ultrahigh temperature pasteurization by direct steam injection (UHT-DSI): (A) unheated 3.4% protein, (B) unheated 7.5% protein, (C) unheated 10.5% protein, (D) UHT-DSI 3.4% protein, (E) UHT-DSI 7.5% protein, and (F) UHT-DSI 10.5% protein.
beverages at different protein concentrations are shown in Table 4. The L-value (whiteness) of the milks was influenced the most by protein level, heat treatment and their interaction and increased with both protein level and heat treatment. The direct effect of protein explained 55% of the variation in L-value and the interaction effect explained 34% of the variation in L-value (Table 3). The increase in whiteness of these MF skim milks with increasing protein concentration was similar to that produced by increasing fat content by 2%. L-value increased with increasing protein concentration due to more reflected light from the higher concentration of casein micelles and removal of soluble light absorbing compounds. Bastian et al. (1991) reported that riboflavin was removed in permeate during UF of milk. Microfiltration would also be expected to remove

**Figure 5 (Continued).** Laser light scattering particle size distribution for low lactose, low serum protein beverages at 3.4, 7.5, and 10.5% protein with no heat treatment and after ultrahigh temperature pasteurization by direct steam injection (UHT-DSI): (A) unheated 3.4% protein, (B) unheated 7.5% protein, (C) unheated 10.5% protein, (D) UHT-DSI 3.4% protein, (E) UHT-DSI 7.5% protein, and (F) UHT-DSI 10.5% protein.

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riboflavin from the retentate and this would explain why the a-value (greenness) of the MF retentate was less than skim milk and the L-value was higher (at all protein concentrations) than skim milk (Table 4). Variation in protein concentration in the fat free MF protein beverages in the current study explained from 55 to 75% of the variation in L, a, and b*-values (Table 3). There was strong heat treatment by protein concentration interaction (Table 3) on L-value that was likely due to the increase in particle size caused by the heat treatment (Figure 5). Previous studies have also indicated casein micelles to be the primary light reflecting particle in skim milk protein beverages (Misawa et al., 2016; Cheng et al., 2019b). Likewise, Lee et al. (2017) and Hoyt et al. (2023) determined whiteness (L value) to increase with heat in milk protein beverages (Lee et al., 2017; Hoyt et al., 2023).

**Beverage Sensory Properties**

The sensory data from the beverages is presented in Tables 5, 6, and 7. Proteins that are thermally degraded to produced sulfur/eggy off flavors were reported to be milk serum proteins (Jo et al., 2018). Vogel et al. (2021) reported 92% casein purity MCC to have no detectable sulfur/eggy off flavors caused by thermal denaturation of SP, indicating most SP has been removed. No sulfur-eggy flavor was detected in any of the MCC beverages either before or after UHT-DSI treatment in our study. This was expected due to the high proportion of SP removal by MF (Figure 2) and high CNTP (Table 1), which was consistent with the results of Whitt et al. (2022) for serum protein removal and for MF milk protein beverages produced by Hoyt et al. (2023). In general, the studies by Hernandez (2023) and Truong (2024) that used UF to remove lactose and soluble mineral reported that the resulting UF beverages before heat treatment were bland and more opaque and white than skim milk and the overall sensory characteristics of the MF protein beverages before thermal processing in the current study were also more white and more bland than the starting skim milk.

Sensory opacity was influenced by both protein concentration (Table 6) and heat treatment (Table 7) with protein concentration having a larger relative impact (i.e., 62% of explained variation) on opacity (Table 5). Sensory opacity results are consistent with previous results in UHT-DSI processed 7.5% protein MCC (Hoyt et al., 2023). Likewise, increased protein content of milk protein beverages has been demonstrated to increase sensory opacity (Cheng et al., 2019b; Hernandez et al., 2023; Hoyt et al., 2023). Only small differences in sensory whiteness were observed (Tables 6 and 7). Previous studies have reported sensory opacity to increase with increasing protein concentration in both UF (Quinones et al., 2018) and MF (Hoyt et al., 2023).

### Table 3. Relative percentage of ANOVA type III sum of squares for color (L, a, and b* values), apparent viscosity (AV), and volume mean diameter [D (4,3)] for the effect of heat treatment, protein concentration, replicate and interactions

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>L</th>
<th>a</th>
<th>b*</th>
<th>AV</th>
<th>D (4,3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>54.96</td>
<td>76.39</td>
<td>75.10</td>
<td>91.80</td>
<td>0.65</td>
</tr>
<tr>
<td>Heat treatment</td>
<td>8.71</td>
<td>23.26</td>
<td>21.08</td>
<td>2.51</td>
<td>98.48</td>
</tr>
<tr>
<td>Replicate</td>
<td>0.14</td>
<td>0.35</td>
<td>0.54</td>
<td>0.48</td>
<td>0.10</td>
</tr>
<tr>
<td>Protein x heat treatment</td>
<td>33.73</td>
<td>NS</td>
<td>3.02</td>
<td>3.03</td>
<td>0.64</td>
</tr>
<tr>
<td>Protein x replicate</td>
<td>2.45</td>
<td>NS</td>
<td>0.26</td>
<td>2.17</td>
<td>0.12</td>
</tr>
<tr>
<td>Heat treatment x replicate</td>
<td>2.45</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Sum</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

1NS = not significant.

### Table 4. L, a, and b* values of low lactose and low serum protein microfiltered heat processed milk protein beverages measured at 4°C

<table>
<thead>
<tr>
<th>Target % protein</th>
<th>L</th>
<th>a</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4</td>
<td>80.71</td>
<td>−3.14</td>
<td>−2.32</td>
</tr>
<tr>
<td>7.5</td>
<td>83.79</td>
<td>−2.10</td>
<td>0.64</td>
</tr>
<tr>
<td>10.5</td>
<td>83.45</td>
<td>−1.87</td>
<td>0.43</td>
</tr>
</tbody>
</table>

a, b, c Means within a column with different superscripts differ (P < 0.05).
et al., 1997) and in MF beverages with both increasing protein concentration and CN%TP (Cheng et al., 2018). There was a protein concentration x heat treatment interaction ($P < 0.05$) for both L-value (Table 3) and sensory whiteness (Table 5) with the interaction of protein concentration x heat treatment interaction having a much stronger impact on L-value (Table 3). These properties of the beverages would be influenced by both increased particle size (more light scattering), removal of light absorbing compounds in the permeate during microfiltration, and heat induced chemical reactions caused by the UHT-DSI treatment. Similarly, Hernandez et al., (2023) reported that removal of lactose and soluble mineral increased milk protein beverage whiteness (Hernandez et al., 2023).

As expected, heat treatment explained most (ca. 86%) of the variation (Table 5) in cooked/milky flavor (Table 7), with a smaller increase ($P < 0.05$) in cooked/milky flavor with increased protein concentration (Table 6). Increased cooked/milky flavor with increased heat treatment has been reported in many studies (Lee et al., 2017; Vogel et al., 2021; Jo et al., 2018). Heat treatment and higher protein concentration increased astringent mouthfeel (Tables 6 and 7) with a protein concentration x heat treatment interaction (Table 5). The large particle size observed in the 7.5 and 10.5% formulations (Figures 5 D, E, F) are consistent with the chalkiness and astringency detected by sensory panelists. These results are supported by previous research that documented chalkiness in 10.5% protein 50:50 SPI:MPC beverages (Vogel et al., 2021). Large particles

---

**Table 5.** Relative percentage of ANOVA type III sum of squares for sensory analysis descriptors for the effect of heat treatment (heat), protein concentration (prot), panelist (pan), replicate (rep) and their interactions

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Opacity</th>
<th>Whiteness</th>
<th>Overall Aroma</th>
<th>Cooked milky</th>
<th>Astringent mouthfeel</th>
<th>Viscosity</th>
<th>Chalky</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prot</td>
<td>62.22</td>
<td>46.00</td>
<td>32.53</td>
<td>11.43</td>
<td>29.79</td>
<td>81.53</td>
<td>9.10</td>
</tr>
<tr>
<td>Heat</td>
<td>35.63</td>
<td>2.50</td>
<td>6.26</td>
<td>85.75</td>
<td>52.82</td>
<td>4.44</td>
<td>80.60</td>
</tr>
<tr>
<td>Pan</td>
<td>0.51</td>
<td>8.28</td>
<td>2.35</td>
<td>0.92</td>
<td>1.28</td>
<td>0.61</td>
<td>0.16</td>
</tr>
<tr>
<td>Rep</td>
<td>0.01</td>
<td>1.24</td>
<td>0.64</td>
<td>0.62</td>
<td>0.35</td>
<td>1.97</td>
<td>0.30</td>
</tr>
<tr>
<td>Prot*heat</td>
<td>0.95</td>
<td>13.50</td>
<td>58.23</td>
<td>0.69</td>
<td>11.70</td>
<td>5.05</td>
<td>9.10</td>
</tr>
<tr>
<td>Prot*pan</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>6.41</td>
<td>NS</td>
</tr>
<tr>
<td>Prot*rep</td>
<td>0.68</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Heat*pan</td>
<td>NS</td>
<td>11.74</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Heat*rep</td>
<td>NS</td>
<td>1.24</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Pan*rep</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Prot<em>Heat</em>pan</td>
<td>NS</td>
<td>6.89</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Prot<em>Heat</em>rep</td>
<td>NS</td>
<td>4.51</td>
<td>NS</td>
<td>NS</td>
<td>2.42</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Heat<em>pan</em>rep</td>
<td>NS</td>
<td>4.11</td>
<td>NS</td>
<td>1.19</td>
<td>NS</td>
<td>NS</td>
<td>0.22</td>
</tr>
<tr>
<td>SUM</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

$^{1}$NS = not significant.

---

**Table 6.** Means for sensory descriptors (15-point intensity scale) by beverage protein concentration for 7 panelists

<table>
<thead>
<tr>
<th>Target % protein</th>
<th>Opacity</th>
<th>Whiteness</th>
<th>Overall Aroma</th>
<th>Cooked milky</th>
<th>Astringent Mouthfeel</th>
<th>Viscosity</th>
<th>Chalky</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4</td>
<td>12.5$^a$</td>
<td>14.2$^a$</td>
<td>1.1$^b$</td>
<td>3.1$^b$</td>
<td>2.3$^b$</td>
<td>2.6$^b$</td>
<td>0.5$^b$</td>
</tr>
<tr>
<td>7.5</td>
<td>13.2$^a$</td>
<td>13.8$^b$</td>
<td>1.0$^b$</td>
<td>3.4$^a$</td>
<td>2.4$^b$</td>
<td>2.2$^a$</td>
<td>0.6$^a$</td>
</tr>
<tr>
<td>10.5</td>
<td>13.6$^b$</td>
<td>13.7$^b$</td>
<td>1.3$^a$</td>
<td>3.5$^a$</td>
<td>2.7$^a$</td>
<td>2.4$^a$</td>
<td>0.9$^a$</td>
</tr>
</tbody>
</table>

$^{a-b}$ Means within protein concentrations with different superscripts differ ($P < 0.05$).

---

**Table 7.** Means for sensory descriptors (15 point intensity scale) by beverage heat treatment for seven panelists

<table>
<thead>
<tr>
<th>Heat treatment$^1$</th>
<th>Opacity</th>
<th>Whiteness</th>
<th>Overall Aroma</th>
<th>Cooked milky</th>
<th>Astringent Mouthfeel</th>
<th>Viscosity</th>
<th>Chalky</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH</td>
<td>12.7$^b$</td>
<td>13.8$^b$</td>
<td>1.1$^b$</td>
<td>2.9$^b$</td>
<td>2.3$^b$</td>
<td>2.1$^b$</td>
<td>ND$^2$</td>
</tr>
<tr>
<td>UHT-DSI</td>
<td>13.4$^a$</td>
<td>13.9$^a$</td>
<td>1.2$^a$</td>
<td>3.8$^a$</td>
<td>2.7$^a$</td>
<td>2.2$^a$</td>
<td>1.2</td>
</tr>
</tbody>
</table>

$^1$NH = No heat treatment, UHT-DSI = ultra-high temperature pasteurization by direct steam injection.  
$^2$ND = not detected.  
$^{a-b}$ Means within protein concentrations with different superscripts differ ($P < 0.05$)
have been hypothesized to be the cause of astringency due to lubrication reduction and increased frictional properties within beverages, which supports sensory astringency results (De Wijk and Prinz, 2006). Li et al. (2018) recorded increased astringency in UP milks vs HTST milks (Li et al., 2018) and Vogel et al. (2021) determined beverage astringency to be primarily affected by protein amount and ratio of CN to SP (Vogel et al., 2021). Likewise, Cheng et al. (2019b) determined astringency increased with true protein concentration in milk protein beverages (Cheng et al., 2019b).

Sensory viscosity increased ($P < 0.05$) with increasing protein concentration (Table 5 and 6) and this was consistent with AV measurements (Figure 6 and Tables 3). Previous studies also documented a positive correlation between increasing protein concentration and increasing viscosity (Vogel et al., 2021; Cheng et al., 2019b; Lutz et al., 2009). Chalky mouthfeel was detected in beverages that received UHT-DSI thermal treatment but not in the unheated beverages (Table 7), which is consistent with literature documenting chalky mouthfeel in UHT-DSI processed high protein milk beverages (Vogel et al., 2021). Higher sensory chalkiness may be related to the large (10–100 micron) particles in UHT-DSI processed 7.5 and 10.5% protein beverages (Vogel et al., 2021). Higher sensory chalkiness may be related to the large (10–100 micron) particles in UHT-DSI processed 7.5 and 10.5% protein beverages. Heat treatment and a heat treatment x protein concentration interaction explained ($P < 0.05$) nearly 90% of the variation in chalkiness in the beverages. The UHT-DSI thermal processed beverages had more cooked milky, chalky, viscosity and astringent mouthfeel than the unheated beverages and those characteristics were more pronounced as protein concentration increased.

This study reports an alternative process to produce low lactose or lactose free protein beverages while also reducing calories (removing lactose in place of enzymatic hydrolysis). The application of microfiltered skim milk with reduced serum protein in place of ultrafiltered skim milk also provides an approach to eliminate undesirable sulfur/eggy flavors in UHT-DSI processed shelf stable or ESL protein beverages. Future work should address consumer perception of these products.

**CONCLUSIONS**

Microfiltration of skim milk at 50°C using 0.1 micron ceramic membranes and diafiltration ratio of water to milk of about 2.5 was able to simultaneously achieve a high level of removal of milk lactose, serum proteins, and soluble minerals to produce protein beverages containing from 3.4 to 10.5% true protein from skim milk. The soluble mineral plus lactose content was very low and the aqueous phase of the beverages had a freezing point very close to water (i.e., $-0.02^\circ$C). Beverage pH ranged from 7.19 to 7.41, with pH decreasing with increasing protein concentration. Overall, the beverages were more white and bland than skim milk. When UHT processed with direct steam injection at a holding temp of 140°C for 2 to 3 s, there was protein aggregation detected by particle size analysis (volume mean diameter 0.16 micron before and 22 microns after UHT). No sulfur eggy flavor was detected and no browning

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**Figure 7.** Principal component analysis (PCA) biplot of sensory descriptors for skim milk, unheated (NH) and ultrahigh temperature direct steam injection processed (UHT-DSI) low serum protein and low lactose beverages containing 3 different protein concentrations (3.4, 7.5, 10.5%).
was observed due to the UHT. Both apparent viscosity and sensory viscosity increased with increasing protein concentration and heat treatment.

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